APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2004, p. 6363–6369 Vol. 70, No. 11
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.11.6363–6369.2004

Dynamics of a Pasture Soil Microbial Community after Deposition of Cattle Urine Amended with [^{13}C]Urea

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Received 10 March 2004/Accepted 22 June 2004

Within grazed pastures, urine patches are hot spots of nitrogen turnover, since dietary N surpluses are excreted mainly as urea in the urine. This short-term experiment investigated ^{13}C uptake in microbial lipids after simulated deposition of cattle urine at 10.0 and 17.1 g of urea C m^{-2}. Confined field plots without or with cattle urine amendment were sampled after 4 and 14 days, and soil from 0- to 5-cm and 10- to 20-cm depths was analyzed for content and composition of phospholipid fatty acids (PLFAs) and for the distribution of urea-derived ^{13}C among individual PLFAs. Carbon dioxide emissions were quantified, and the contributions derived from urea were assessed. Initial changes in PLFA composition were greater at the lower level of urea, as revealed by a principal-component analysis. At the higher urea level, osmotic stress was indicated by the dynamics of cyclopropane fatty acids and branched-chain fatty acids. Incorporation of ^{13}C from [^{13}C]urea was low but significant, and the largest amounts of urea-derived C were found in common fatty acids (i.e., 16:0, 16:1ω7*c*, and 18:1ω7) that would be consistent with growth of typical NH_4^+-oxidizing (*Nitrosomonas*) and NO_2^--oxidizing (*Nitrobacter*) bacteria. Surprisingly, a 20‰ depletion of ^{13}C in the cyclopropane fatty acid cy17:0 was observed after 4 days, which was replaced by a 10 to 20‰ depletion of that in cy19:0 after 14 days. Possible reasons for this pattern are discussed. Autotrophic nitrifiers could not be implicated in urea hydrolysis to any large extent, but PLFA dynamics and the incorporation of urea-derived ^{13}C in PLFAs indicated a response of nitrifiers which differed between the two urea concentrations.

Urine deposition is an important source of N in grazed pastures. Nitrogen turnover in urine patches is intense, and the potential for environmental losses is significant, depending on urine composition (31, 49). Excess N in the diet of cattle is mainly excreted as urea in the urine (23), and understanding the regulation of urea turnover in urine patches is therefore of particular interest. Urea typically disappears from the soil solution within 24 to 48 h and is replaced by high concentrations of NH_4^+ (18, 36). The accompanying increase in osmotic pressure and free NH_3 concentrations can lead to N_2O emissions and other microbial stress reactions (12, 40).

Urea hydrolysis to NH_4^+ and CO_2 may occur both on plant surfaces and in the soil (20). Hydrolysis takes place through the action of free or colloid-bound extracellular ureases or intracellularly following microbial uptake (20, 39). Klose and Tabatabai (26) determined extracellular and intracellular urease activities to be on average 54 and 46% of the total potential activity in various cropping systems. Nielsen et al. (33) found that urea turnover was comparable in magnitude to gross N mineralization and hypothesized that direct uptake and intracellular metabolism of urea are quantitatively important aspects of N cycling in agricultural soil. Different bacteria have shown a capacity for urea uptake, including *Klebsiella* and *Alcaligenes* spp. (21) and autotrophic NH_4^+-oxidizing bacteria (2).

Stable-isotope analysis of microbial lipids has recently been introduced in community-level studies. Even though isotope fractionation during lipid biosynthesis is a matter of concern (5), this approach can potentially link specific substrates with individual populations in situ (6, 16, 22). Substrate-derived ^{13}C has been traced in membrane lipids of heterotrophic (1, 7) and autotrophic (27) organisms. Further, ^{13}C enrichment of phospholipid fatty acid (PLFA) profiles has been used to investigate microbial community dynamics (9).

Here we report on the turnover of [^{13}C]urea in pasture soil and the associated incorporation of ^{13}C in PLFAs after simulated deposition of cattle urine at two different urea concentrations. It was hypothesized that the dynamics and labeling of PLFAs could provide information about the fate of urea and the response of nitrifiers to the sudden increase in N availability.

MATERIALS AND METHODS

Site information. The field experiment was carried out between 20 September and 4 October 2001. The site was an 8-year-old grass-clover pasture near the Danish Institute of Agricultural Sciences, Tjele, Denmark (55°52′N, 9°34′E). The soil was a sandy loam and was classified as a Typic Hapludult. It contained 2.7% C and 0.18% N, the pH_{CaCl_2} was 5.5, and total cation exchange capacity was 8.7 cmol kg^{-1}. Air and soil temperatures (10-cm depth) averaged 12.8 and 13°C, respectively, during the experiment, and total precipitation was 56 mm (27 mm within the first 48 h).

Experimental design. The experimental design contained three randomized blocks, each with three treatments, i.e., a control with no amendments (CTL), standard cattle urine (LU), and urine with approximately double the urea concentration (HU). Urine was collected from dairy cows during milking 3 days before start of the experiment and was stored at 2°C until used; the urea content was determined prior to storage. On day 0 of the experiment, the cattle urine was amended with [^{13}C]urea (99 atom%; CK Gas Products, Hook, United Kingdom) and unlabeled urea (HU treatment only), to give concentrations of 12.0 and 20.4 g of urea liter^{-1} with [^{13}C]urea concentrations of 11.0 atom% (LU) and 18.3 atom% (HU). The labeled urine was applied to 25- by 35-cm frames, installed to

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a 15-cm depth, at rates corresponding to 10.0 and 17.1 g of urea C m^{-2} in for the LU and HU treatments, respectively.

Sampling. Emissions of CO_2 were determined after 0.2, 1, 2, 4, 6, and 14 days. Insulated chambers, equipped as previously described (35), were mounted on top of the permanent frames. Gas samples (13 ml) were taken in preevacuated Exetainers (Labco Ltd., High Wycombe, United Kingdom) after 5, 15, and 45 min and subsequently analyzed for total concentrations and isotopic composition of CO_2.

Soil sampling took place after 4 and 14 days. Three soil cores (0 to 20 cm in depth; 2 cm in diameter) were collected and the depth intervals 0 to 5, 5 to 10, and 10 to 20 cm were pooled in separate bags. The samples were stored at 2°C until sieved (<4 mm) and were processed within 2 days. Subsamples were extracted in 1 M KCl and analyzed for urea, NH_4^+, and NO_2^- plus NO_3^- (25, 32). From two of the three blocks, 3- to 3.5-g subsamples from 0- to 5-cm and 10- to 20-cm depths (selected samples) were prepared for PLFA analysis by using a modified Bligh-Dyer single-phase extraction, solid-phase extraction on 100-mg SPE columns (Varian, Harbor City, Calif.), and mild alkaline transesterification as previously described (37).

Soil was dried at 105°C overnight for determination of gravimetric soil moisture. Dried subsamples were analyzed for total C and ^{13}C. The soil bulk density of each depth interval was determined by the end of the experiment.

GC-IRMS analyses. The concentrations and isotopic composition of CO_2 were analyzed with a Europa (Crewe, United Kingdom) Scientific Tracermass isotope ratio mass spectrometer (IRMS) coupled to an automated gas analysis system. The total C content and isotopic composition of soil samples were determined by using an automated combustion elemental analyzer interfaced with a Europa ANCA-SL IRMS system. ^{13}C-labeled PLFA methyl esters were analyzed on a Finnigan Delta Plus XL gas chromatograph (GC)-combustion IRMS (Thermo-Quest, Pegnitz, Germany). The gas chromatograph (Hewlett-Packard 6890) was equipped with an HP-5MS column (60 m by 0.25 mm [inner diameter]) and a GC/C III combustion interface. Helium was used as carrier gas.

Fatty acids were tentatively identified from retention times and cross-referencing with samples analyzed by GC-MS. The $\delta^{13}C$ values determined by GC-combustion IRMS, based on authentic standards certified relative to PeeDee Belemnite, were corrected for the isotope ratio of the methyl moiety of fatty acid methyl esters (1), as follows: $\delta^{13}C_{FA} = [(C_n + 1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/C_n$, where $\delta^{13}C_{FA}$ is the $\delta^{13}C$ of the fatty acid, C_n is the number of C atoms in the fatty acid, and $\delta^{13}C_{FAME}$ is the $\delta^{13}C$ of the fatty acid methyl ester. The fractions of ^{13}C in each fatty acid and the amounts of ^{13}C incorporated were calculated as outlined by Boschker and Middelburg (5).

Statistical analyses. Concentrations of urea, NH_4^+, and NO_2^- plus NO_3^-, respectively, were compared across treatment, sampling day and depth by a linear mixed model, as follows: $Y = \mu + \alpha_S + \beta_T + (\alpha\beta)_{ST} + \gamma_D + (\alpha\gamma)_{SD} + (\beta\gamma)_{TD} + (\alpha\beta\gamma)_{STD} + \epsilon_B + E_{TB} + E'_{STB} + E''_{DTB} + E'''STDB$, where Greek letters represent treatment effects, S is sampling day, T is urine treatment, D is depth interval, B is block, and E terms represent random errors. Nitrogen concentrations were log transformed to reduce heteroscedacity.

The PLFA distributions were analyzed by a principal component analysis (PCA) after $\log(n + 1)$ transformation of moles percentages and with the covariance matrix. The concentrations of individual fatty acids at the 0- to 5-cm depth were also compared across treatments and sampling days by individual analyses of variance (ANOVAs), using the stepwise Bonferroni procedure to control the overall table-wise error rate (41). Statistical analyses were carried out with SAS 8.2 (SAS Institute, Cary, N.C.).

RESULTS

CO_2 emissions and ^{13}C recovery. The cumulated emissions of CO_2 from soil and vegetation during the 14-day period (average ± standard error) amounted to 19.8 ± 2.9, 25.8 ± 6.0, and 22.6 ± 1.8 g of C m^{-2} in the CTL, LU, and HU treatments, respectively, and were not statistically different between treatments. In both the LU and HU treatments, the proportion derived from urea constituted around 35% of the total flux at the first sampling after 3 h, a proportion which decreased to <1% by day 4 and to 0.1% by day 14. The cumulated recovery of ^{13}C in CO_2 by day 14 was 15 ± 9.2% in the LU treatment and 7.7 ± 2.0% in the HU treatment. The recovery of ^{13}C in the soil was 37 ± 2.6% in the LU treatment and 16 ± 1.0% in the HU treatment. Finally, the recovery of ^{13}C in PLFAs was much less than 1% in both treatments (see below).

TABLE 1. Concentrations of extractable nitrogen at three soil depth intervals 4 and 14 days after simulated urine deposition[a]

Compound and depth (cm)	μg of N cm^{-3}					
	Day 4			Day 14		
	CTL	LU	HU	CTL	LU	HU
Urea						
0–5	0.9a	1.1ab	4.0c	1.5ab	2.3ab	3.3bc
5–10	0.3a	0.4a	0.4a	0.3a	0.5a	1.4a
10–20	0.1a	0.2a	0.2a	5.0b	0.0a	0.3a
Ammonium						
0–5	2.6a	278c	527c	2.6a	75b	325c
5–10	1.4a	103c	140c	1.6a	42b	99c
10–20	0.2a	18c	36c	0.5a	6.7b	17c
Nitrite + nitrate						
0–5	0.5a	5.3b	9.2b	0.5a	33c	46c
5–10	0.5a	4.5b	6.7b	1.0a	33c	35c
10–20	0.3a	3.7b	5.4b	0.5a	19c	27c

[a] Effects of treatment, sampling day, and depth interval were tested with a linear mixed model (see text). Only differences between treatments and sampling days (within row) are shown. Letters a, b, and c indicate whether differences are significant ($P < 0.05$; $n = 3$).

Soil nitrogen dynamics. Background concentrations of extractable N in the pasture soil were very low, and N introduced via simulated urine deposition was therefore readily detected (Table 1). There were strong vertical gradients, with more than half of the extractable N in the top 5 cm. By day 4, urea concentrations were low, yet they were elevated at the 0- to 5-cm depth in the HU treatment relative to the other treatments. The NH_4^+ pool remaining in the soil by day 14 was significantly higher in the HU treatment than in the LU treatment at all depths. Accumulation of NO_2^- plus NO_3^- occurred at similar rates in the LU and HU treatments and by day 14 corresponded to 23 and 17%, respectively, of the urea N added.

PLFA content and composition. Only the 13 phospholipid fatty acids shown in Table 2 were consistently detected in all treatments and on both sampling days. Table 2 presents the moles percent distributions and total yields at the 0- to 5-cm soil depth. Urine deposition resulted in PLFA concentrations increasing from 26.6 to 36 to 39 nmol g^{-1}, an increase that was maintained in the HU treatment, but not in the LU treatment, by day 14. The need to control type 1 errors made the statistical tests of individual PLFAs relatively conservative. Accordingly, only a few significant effects were identified (Table 2), the most notable being the reduction of cy17:0/16:1ω7 ratios after 4 days. There was a net production of cyclopropane fatty acids (CFAs) in the HU treatment by day 4.

A PCA based on all treatments and both depths (selected samples from 10- to 20-cm depth only) separated the two depth intervals along the first principal component (Fig. 1A). The samples from the 10- to 20-cm depth were enriched in cy17:0 and cy19:0, in 10Me16:0, and in 18:0 (Fig. 1B). At the 0- to 5-cm depth, the PLFA profiles of the CTL and HU treatments were similar after 4 days. In contrast, the PLFA composition of the LU treatment had changed dramatically, but it shifted

TABLE 2. Distribution of PLFAs, total yields of PLFAs, and cyclopropane fatty acid-to-precursor ratios at the 0- to 5-cm soil depth in the CTL, LU, and HU treatments

Parameter and PLFA	Mean (SD)[a]						F^b
	4 days			14 days			
	CTL	LU	HU	CTL	LU	HU	
mol%							
i15:0	5.4 (0.3)	7.5 (0.7)	4.7 (0.0)	6.6 (1.6)	6.8 (0.6)	6.8 (0.4)	3.47
a15:0	5.4 (0.3)	7.8 (1.5)	6.8 (0.2)	5.5 (1.5)	6.4 (0.5)	6.5 (1.0)	1.56
i16:0	1.9 (0.1)	3.2 (0.3)	1.8 (0.1)	3.1 (0.4)	2.0 (0.2)	2.1 (0.3)	12.1*
16:1ω7 c	7.7 (1.8)	11.3 (1.3)	10.5 (0.7)	8.2 (0.1)	11.2 (0.2)	13.3 (1.8)	5.79
16:1ω5 c	7.3 (0.0)	6.7 (0.5)	6.9 (0.6)	6.9 (0.4)	7.8 (1.0)	6.7 (1.3)	0.67
16:0	21.8 (0.3)	19.1 (0.2)	18.0 (2.2)	18.3 (1.4)	19.4 (0.4)	18.4 (0.1)	3.37
10Me16:0	2.8 (0.5)	2.3 (0.1)	3.5 (0.2)	4.1 (1.0)	2.8 (0.2)	3.2 (0.4)	3.1
cy17:0	3.0 (0.8)	1.9 (0.2)	3.3 (0.2)	3.2 (0.4)	3.1 (0.4)	3.0 (0.2)	3.07
18:2ω6 c	3.3 (0.6)	5.0 (0.4)	2.3 (0.3)	4.8 (1.9)	2.7 (0.8)	3.5 (1.3)	2.19
18:1ω9 c	12.0 (1.5)	11.8 (0.7)	12.0 (0.5)	12.8 (0.0)	12.0 (0.4)	11.6 (0.5)	0.56
18:1ω7 c	20.4 (2.2)	18.4 (1.2)	22.8 (0.2)	18.0 (3.1)	20.8 (0.3)	19.9 (1.7)	2.00
18:0	3.0 (0.3)	1.5 (0.1)	2.4 (0.0)	3.2 (0.6)	2.4 (0.2)	2.2 (0.1)	8.84*
cy19:0	6.1 (0.4)	3.4 (0.4)	5.0 (1.1)	5.5 (2.2)	2.5 (0.1)	2.8 (0.2)	4.1
Yield (nmol g^{-1})	26.6 (0.02)	38.7 (3.0)	35.8 (2.8)	23.5 (4.9)	27.4 (0.2)	36.9 (0.3)	11.96*
Ratio							
cy17:0/16:1ω7	0.38 (0.01)	0.17 (0.00)	0.31 (0.00)	0.39 (0.04)	0.28 (0.04)	0.22 (0.02)	23.77**
cy19:0/18:1ω7	0.30 (0.05)	0.19 (0.04)	0.22 (0.05)	0.30 (0.07)	0.12 (0.00)	0.14 (0.02)	5.84
i15:0/a15:0	0.99 (0.003)	0.97 (0.07)	0.69 (0.01)	1.21 (0.03)	1.05 (0.001)	1.06 (0.17)	5.39

[a] $n = 2$. F values from individual ANOVAs were corrected according to the stepwise Bonferroni procedure.
[b] *, $P < 0.05$; **, $P < 0.01$.

towards the composition of the CTL treatment after 14 days. The PLFA profile of the HU treatment changed in the same direction as that of the LU treatment between day 4 and day 14.

$\delta^{13}C$ signature of PLFAs. The recovery of urea-derived ^{13}C in PLFAs was small, resulting in $\delta^{13}C$ changes of not more than 20‰ (Fig. 2, top panels). Incorporation of ^{13}C was observed in branched-chain fatty acids (i15:0, a15:0, and i16:0), in C_{16} straight-chain fatty acids (mainly 16:0 and 16:1ω7), and in C_{18} straight-chain fatty acids (18:0, 18:1ω9, and 18:1ω7). Interestingly, a decrease in $\delta^{13}C$ was observed with both cy17:0 (LU treatment by day 4) and cy19:0 (LU and HU treatments by day 14). Even though the changes in concentrations of CFAs were moderate, the $\delta^{13}C$ depletion thus revealed that these CFAs were characterized by significant turnover. At the 0- to 5-cm soil depth, the $\delta^{13}C$s of CFAs in the LU and HU treatments were on average 13 to 15‰ lower than the average $\delta^{13}C$ for all other PLFAs by day 4 and were 7 to 12‰ lower by day 14. Cyclopropane fatty acids in the CTL treatment were also depleted of ^{13}C, with a $\delta^{13}C$ of 2 to 5‰ below the average for all other PLFAs (data not shown).

Recoveries of urea-derived C in PLFAs after 4 days were comparable in the HU and LU treatments, at 0.001 and 0.0009%, respectively. The concentrations of urea C incorporated in individual PLFAs are presented in Fig. 2, bottom panels. These data show a selectivity of ^{13}C incorporation between and within treatments; six fatty acids (i15:0, a15:0, 16:1ω7, 16:0, 18:1ω9, and 18:1ω7) accounted for 85 to 95% of the total ^{13}C incorporation. These fatty acids also accounted for most of the PLFA concentration changes in urine-amended soil relative to the CTL treatment. The LU and HU treatments differed with respect to the incorporation of urea C into the branched-chain fatty acids *i*15:0 and *a*15:0, resulting in low *i*15:0/*a*15:0 ratios in the HU treatment by day 4 (Table 1). The absence of excess ^{13}C in the common fatty acid 16:0 in the LU treatment after 4 days is notable, but it cannot be explained at present.

There was a significant ($P < 0.01$ or better) positive relationship between total concentration changes and the incorporation of ^{13}C in individual PLFAs. In Fig. 3, the average fractions of PLFA carbon derived from urea in the LU and HU treatments after 4 and 14 days are plotted against the fractions of CO_2 derived from urea. On day 14, the incorporations of urea C in CO_2 and PLFAs were relatively similar in the LU and HU treatments (close to the 1:1 line). In contrast, on day 4, the labeling of excess PLFA was greater than the labeling of CO_2 emitted, especially in the HU treatment.

DISCUSSION

Urine patches in grazed pastures constitute a harsh environment that is potentially stressful for soil organisms. Scorching of the vegetation is known to occur, depending on deposition rate and urea concentration (42), and it is a result of osmotic stress, NH_3 toxicity, or a combination of both (18, 31, 42). Plants and microbes respond similarly to hyperosmotic conditions (11), and adverse effects on microbial populations can therefore also occur. Polonenko et al. (40) exposed soil columns to osmotic potentials of −0.5 MPa and below and found a significant decrease in the number of viable cells leached from the salt-stressed columns but a significant increase in viable cell numbers following a period of stress relief. In the present study, extractable NH_4^+ alone corresponded to osmotic

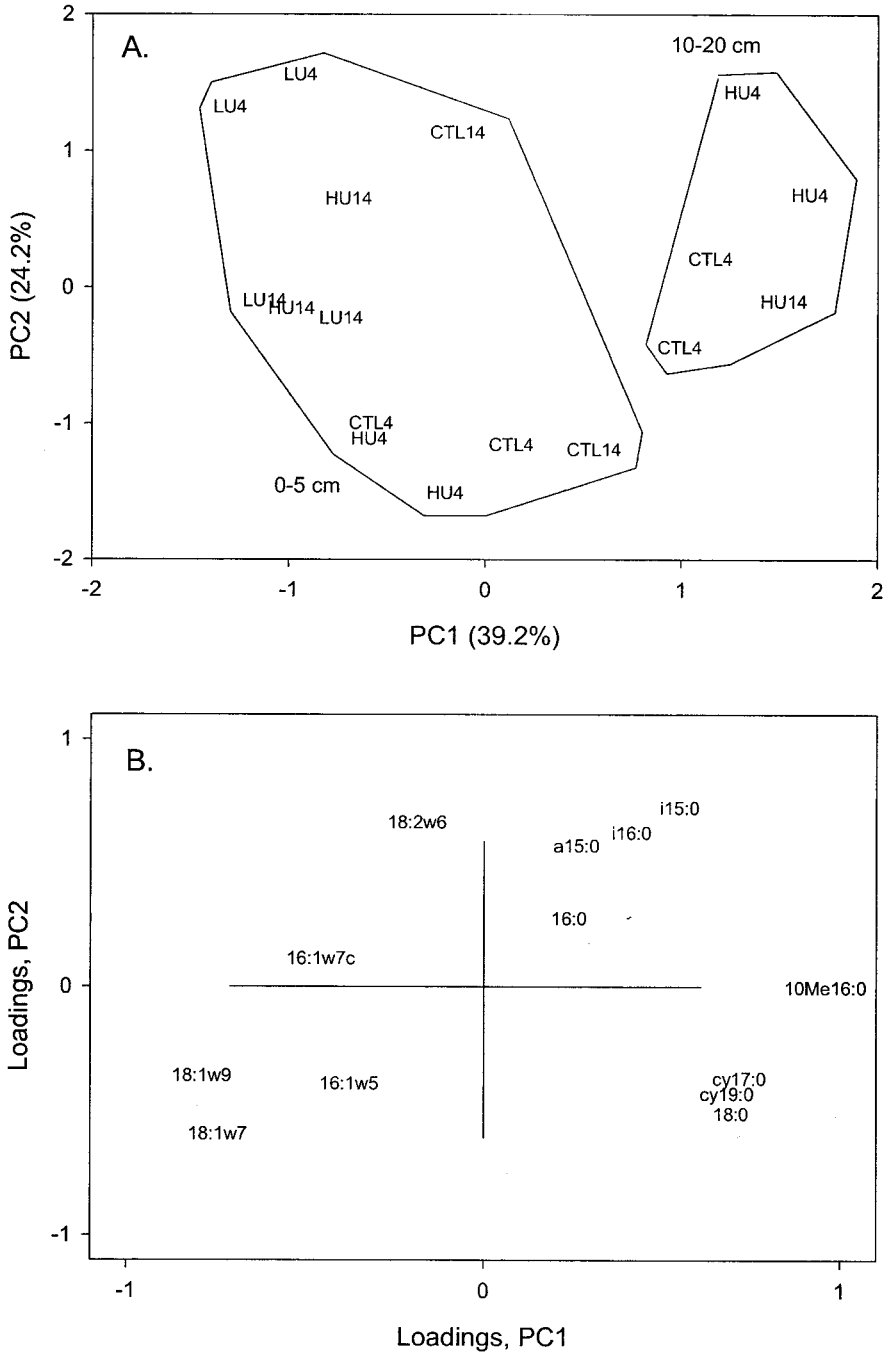

FIG. 1. A. Score plot of the first two principal components of a PCA based on moles percent distributions of PLFAs in pasture soil from the 0- to 5-cm or 10- to 20-cm depth (selected samples only). The designations indicate treatment (CTL, LU, or HU) and sampling day (4 or 14). B. Component loadings of the same PCA analysis.

potentials of down to −0.19 and −0.33 MPa in the LU and HU treatments, respectively.

The urine patch environment is expected to stimulate nitrification, but high urinary N concentrations may also inhibit nitrifying bacteria (12, 31, 38, 47). Nitrite oxidizers such as *Nitrobacter* are far more sensitive to adverse environmental conditions than *Nitrosomonas*, in particular with respect to concentrations of free NH_3 (46). Inhibitory NH_3 levels of 0.1 to 1 and 10 to 150 mg liter^{-1} have been reported for *Nitrobacter* and *Nitrosomonas*, respectively (3). For the HU and LU treatments, concentrations of free NH_3 were estimated from NH_4^+ concentrations and pH to be 80 to 120 and 40 to 65 mg N liter^{-1}, respectively, suggesting that a selective inhibition of NO_2^- oxidation was likely to occur, especially in the HU treatment. Monaghan and Barraclough (31) found that maximum NO_2^- concentrations in urine-amended soil increased progres-

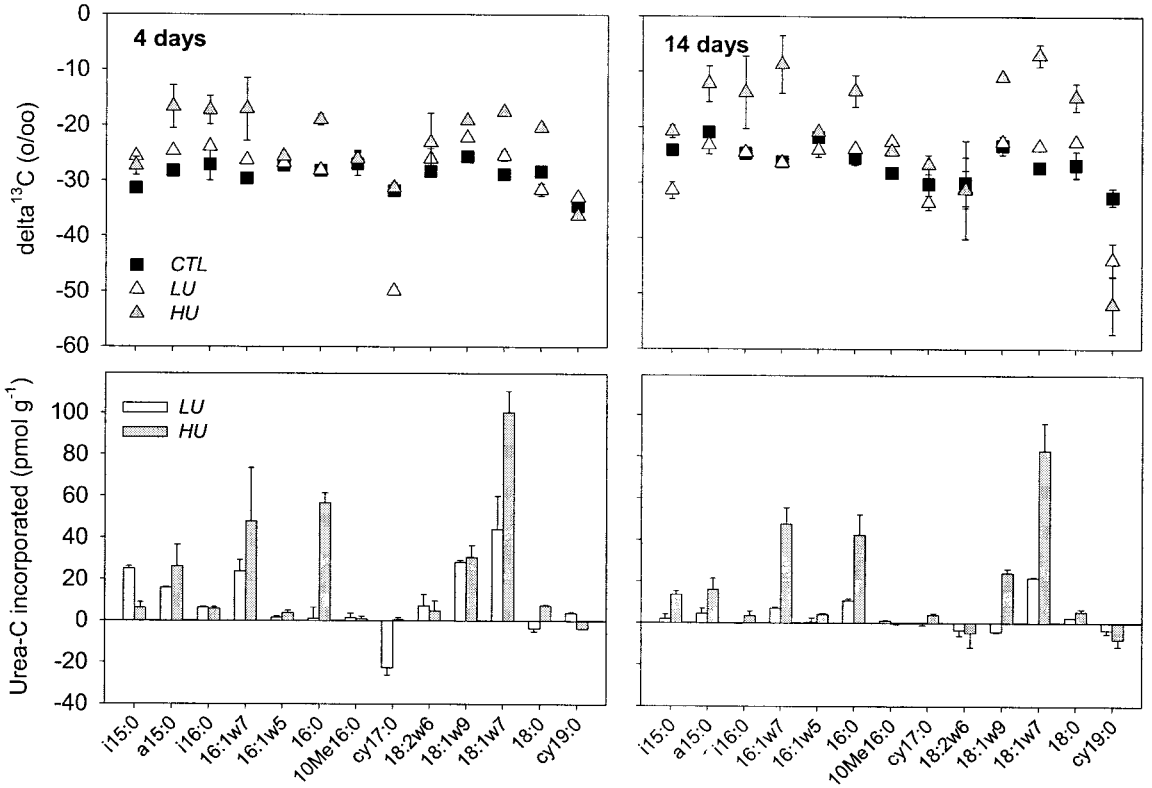

FIG. 2. $\delta^{13}C$ values (top panel) and incorporation of urea-derived C (bottom panel) in PLFAs extracted from pasture soil 4 and 14 days after simulated urine deposition (mean ± standard error; $n = 2$).

sively from 5 to 160 mg of N kg^{-1} soil as urine N increased from 3.8 to 25 g of N liter^{-1}. Nitrite accumulation has also been observed at a urea level corresponding to that in the HU treatment in the pasture soil used in the present study (38).

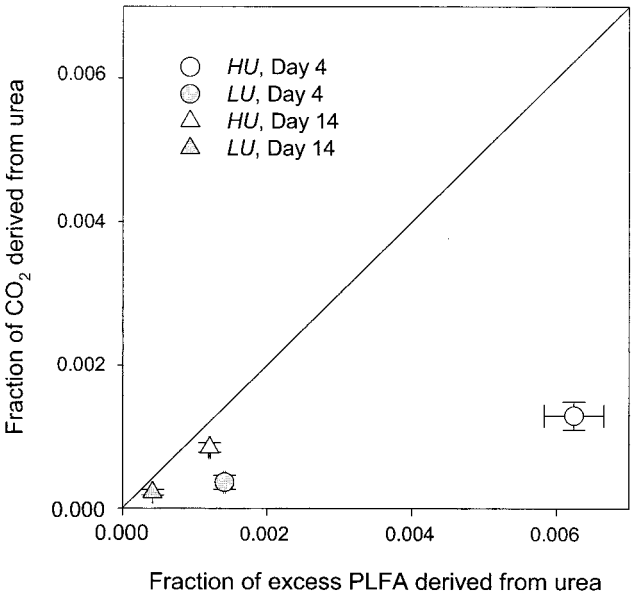

FIG. 3. The fractions of CO_2 derived from urea on day 4 and day 14 were plotted against the fractions of urea C in excess PLFA, i.e., PLFA concentration changes in the LU and HU treatments relative to the CTL treatment (mean ± standard error; $n = 2$).

A response to urine deposition was detectable in the PLFA profiles of the soil microbial community. Significant growth was indicated by the increase in PLFA yields with both the LU and HU treatments relative to the unamended CTL treatment after 4 days (Table 2). This was in accordance with results from the related laboratory study with comparable urea amendments to pasture soil, where a twofold increase in potential NH_4^+ oxidation activity also was observed 3 days after urea amendment (38). In the LU treatment, vigorous growth was further indicated by low CFA-to-precursor ratios (15). In the HU treatment, however, such a reduction in CFA-to-precursor ratios was not observed despite the increase in PLFA yield (Table 2). Instead, a net production of CFAs occurred in the HU treatment, which could be interpreted as a stress response, since cyclopropane fatty acids have been shown to appear in microbial cell membranes in connection with various stresses, including hyperosmotic conditions (14, 29). There is evidence that lipid extractability or partitioning during sample preparation increases with ionic strength (13), but the importance of this factor is not known.

The LU and HU treatments also differed in the proportions of the branched-chain fatty acids i15:0 and a15:0 after 4 days (Fig. 2, bottom left panel). A similar shift towards production of anteiso fatty acids has been observed in response to salt stress with *Listeria monocygotenes* and several halotolerant bacteria (reference 10 and references therein); the resulting increase in membrane permeability was explained as a mechanism to facilitate adaptation to hyperosmotic conditions (10). Reinspection of PLFA results from the laboratory study re-

ferred to above (38) showed a consistent reduction of *i*15:0/*a*15:0 ratios after urea amendment corresponding to the HU treatment but not after urea amendment corresponding to the LU treatment (data available upon request).

As indicated in Fig. 3, the incorporation of urea C in PLFA after 4 days was higher than the proportion of urea C in CO_2, indicating that part of the CO_2 originated from unlabeled substrates in the soil. Stress-induced microbial turnover or degradation of soil organic matter dissolved by the urine could have caused this (24, 38). However, cattle urine contains organic components besides urea, such as hippuric acid (8), and dilution of $^{13}CO_2$ by degradation of these components could also have occurred.

The observed depletion of ^{13}C in CFAs (Fig. 2A) was unexpected but testifies to the turnover of these pools during the experiment. Boschker et al. (6) found cy17:0 to be slightly depleted of ^{13}C after [^{13}C]methane amendment to an intertidal sediment, but ^{13}C depletion of CFAs on the order of 10 to 20‰ has not previously been reported for complex microbial communities. Bacterial CFAs are produced from the monoenes 16:1ω7*c* and 18:1ω7*c* exclusively by *trans*-methylation from *S*-adenosylmethionine (14). Isotope depletion of the active methyl group in *S*-adenosylmethionine by up to 39‰ has been observed in natural compounds other than fatty acids (19, 49), suggesting that the potential for ^{13}C depletion in CFAs is high.

The source of ^{13}C introduced in the experimental system was a C_1 compound, urea. Whereas the isotopic composition of heterotrophs is mostly close to that of the growth substrates (1, 30, 48), the pathways of C assimilation during autotrophic growth can result in ^{13}C depletion of cell material by up to 27‰ (34). Further, lipids are generally depleted in ^{13}C relative to the total biomass (17, 34, 45). Experiments with sulfate-reducing bacteria grown on acetate led to a ^{13}C depletion of 12 and 13‰ in the fatty acid 10Me16:0, whereas growth on CO_2 resulted in depletions of 24 and 18‰ (30). Also, as mentioned above, Boschker et al. (6) found a depletion of ^{13}C in cy17:0 isolated from a sediment microbial community after [^{13}C]methane amendment, but this was not the case after [^{13}C]acetate amendment. Finally, discrimination against ^{13}C during algal lipid biosynthesis was shown to increase with CO_2 availability (43), suggesting that a high soil CO_2 availability in urine patches could also increase isotope fractionation during autotrophic growth.

The actual contribution of nitrifiers to ^{13}C incorporation in PLFAs is not known. The greatest amounts of ^{13}C were found in the common fatty acids 16:0, 16:1ω7*c*, and 18:1ω7*c*, which predominate in *Nitrosomonas* and *Nitrobacter* cell membranes (4, 27, 28) but are also present in many other organisms. Fixation of CO_2 by heterotrophic bacteria may have accounted for some incorporation of ^{13}C into PLFAs, although a more uniform distribution of label within a range of microbial PLFAs would have been expected if this had been the main mechanism for urea C incorporation (44). Both cy17:0 and cy19:0 can be synthesized by autotrophic NO_2^- oxidizers, including *Nitrobacter winogradski* (27, 28). If *Nitrobacter* was the main source of de novo CFA synthesis, then this might explain why ^{13}C depletion was so extreme in these compounds. Future work should address the response of *Nitrobacter* to urine deposition in more detail.

In summary, carbon and nitrogen transformations are intense in pasture soil affected by urine. The response of the pasture soil microbial community was complex. The HU treatment was characterized by osmotic pressures and free NH_3 concentrations which probably caused some stress-induced metabolism and a partial inhibition of nitrification activity during the first few days after deposition (12, 38, 40, 47). The low recovery of urea-derived C in PLFAs did not suggest intracellular urea hydrolysis as a major mechanism for turnover of urinary urea, but still some information about the microbial response to urine deposition was obtained that was not revealed by overall PLFA dynamics. A stress response of nitrifiers to urine deposition was indicated, which differed between the two levels of urinary urea applied.

ACKNOWLEDGMENTS

This study was supported by the EU Framework Programme 5 project MIDAIR (EVK2 CT-2000-00096) and by the Danish Research Centre for Organic Farming.

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